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Distribution of endangered mayfly *Palingenia longicauda* (Olivier, 1791) (Ephemeroptera, Palingeniidae) on the territory of the Republic of Moldova

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With 5 figures and 2 tables

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Schlagwörter: Palingenia, Ephemeroptera, Insecta, Dniester, Prut, Donaz, Moldawien, Biologie, Biomasse, Ökologie, Faunistik

Data on the distribution, frequency, abundance and biomass of *Palingenia longicauda* on the territory of the Republic of Moldova are presented. This species was considered to have disappeared in the 1970ies, however, recently it was rediscovered in 2010 in the Prut River near Cahul. The highest larvae abundance sampled by grabs was 2520 ind/m^2, the calculated maximum of biomass was 109.36 g/m^2. Individual biomass of *P. longicauda* varied between 0,0001 g and 0,358 g and the length of larvae from 5 to 50 mm. Only 30 adult specimens of *P. longicauda* were observed on the water surface at the Dniester River in June 2013.

1 Introduction

Palingenia longicauda is an amphibiotic species, a long-tailed mayfly. The lengths of imago including cerci can reach up to 10 cm, being one of the largest mayflies in Europe (Bauernfeind & Soldán 2012; Soldán et al. 2009).

The life cycle of this species presumably lasts about 3 years (semivoltine, 3Y life cycle): Eggs develop on the bottom of rivers. After about 39-45 days, depending mainly on water temperature (at an average temperature of 21.4 °C it lasts 26 days) larvae hatch from the eggs (Landolt et al. 1997; Tittizer et al. 2008). The larvae live in the substrate for about 3 years (number of larval instars not known), building U-shaped tubes preferably in steep clay banks, in which a water current is created by gill movement (Csoknya & Halasy 1974; Russev 1987). Emergence of subimagines is highly synchronized, the subimaginal stage of males lasting up to 5 minutes. Immediately after moulting the male imagines fly back to the river and begin their "patrolling flight" (Landolt et al. 1995). Females usually remain in the subimaginal stage (interpreted as a case of neoteny by most authors; Bauernfeind & Soldán 2012) and, after copulation, deposit their eggs on the water surface about 3-10 km upstream after a compensation flight (Russev 1987). Eggs of some females (up to 50% according to Landolt et al. 1997) develop parthenogenetically, this being probably relevant for small populations. Period of mass emergence usually lasts about 14 days from end of June until beginning of July.

Palingenia longicauda was widespread in the lower and middle courses of large and medium-sized European rivers until the end of the 19th century (Russev 1987). Historically, fishermen in Moldova used a metal cylinder with a length of 30 cm and a wooden handle for capturing larvae of *Palingenia* as natural bait for fish, the same method as was described by Russev (1987) and Bálint et al. (2012). During the twentieth century industrialization has led to river degradation and water pollution, causing extinction of *P. longicauda* over most of its former area of distribution in Europe (Russev 1987; Soldán et al. 2009). Approximately 98 % of *P. longicauda* populations have been lost during the past century (Bálint et al. 2012). Still vital populations have been recorded only in the Tisza River basin (Soldán et al. 2009; Bálint et al. 2012). Tisza River basin, borders on Prut River and Dniester River basin (JRS map).

The first record of *P. longicauda* from the Dniester River basin on Ukrainian territory was published at the end of the 19th century (Dzięddzielewicz 1867: 161). In the first half of the 20th century this species could be found in the Upper and Middle Dniester (Red Book of Ukraine, 2009), which is a transboundary river for Ukraine and Moldova. On the territory of Moldova first records for Dniester River were published in the monograph "Hydrofauna of Dniester River" on the basis of research results of complex studies carried out during 1946-1956 (Yaroshenko 1957). During 1946-1973 *Palingenia* sp. was considered a common species, being found regularly (Yaroshenko 1957, Mushchinskij 1972). Before 1960, on the river sections with clay banks, *P. longicauda* contributed significantly to the density and biomass of total zoobenthos (Byzgu et al. 1964). In 1961 Dediu & Val'kovskaja (1961) recorded *Palingenia* sp. as a common species for the Prut River. Later, Mushchinskij (1972) cited *P. longicauda* and *P. fuliginosa* from the Prut River near Cahul. The period which followed (1974-2007) was marked by no records of *P. longicauda* at the sampling sites in the Republic of Moldova during regular or occasional field works. This may have been caused by anthropogenic activity such as hydrotechnical constructions and water pollution as in many other European countries. The species is very sensitive towards changes in the environmental conditions (Russev 1987; Landolt et al. 1995) which influence its abundance.

This rare and critically endangered European species has been included in the Appendix II of the Convention on the Conservation of European Wildlife and Natural Habitats (Bern Convention, 1998), in the Carpathian List of Endangered Species (Czech Republic, Romania, Poland, Slovakia, Ukraine and Hungary, 2003) and The Red book of Ukraine (2009). Nevertheless, this species has never been included as endangered on the territory of the Republic of Moldova (The Red book of the Republic of Moldova, 2015).

Recently *P. longicauda* reappeared in some sections of the River Danube and some other rivers, including Prut River, after many years of not having been detected (Málnás et al. 2016). Since the Prut River represents the last large left tributary of the Danube, and belongs to the Wetland Danube River Area, the investigation of the state of this rare species is considered of international importance.

For example, in 2007, during an international longitudinal ship survey JDS2 the entire length of the Danube River was studied, which included a total of 124 sampling sites along an area of 2600 km (including the major tributaries). In this report it was noted that rare species like large burrowing Ephemeroptera: *Palingenia longicauda* were found in the Prut River, close to its confluence with the Danube River (135 km RiverKm MD /Prut (rkm 1.0) RO MD 23/09/07). (ICPDR, 2008). Recently a refuge area for *P. longicauda* in the Danube Delta was identified in Romania (Bulancova et al. 2009).

The aim of the present paper was to inventory all existing data from the previously published literature about the distribution of *P. longicauda* on the territory of Moldova, to investigate the current state of *P. longicauda* on this territory and to analyse some additional data collected from neighboring countries.

2 Material and methods

2.1 Study Area and sampling methods

On the territory of the Republic of Moldova there are four water basins: Dniester River basin, Prut River basin, small rivers of the Danube River Basin and small rivers of the Black Sea basin. The largest rivers in the country are Dniester and Prut (Fig. 1). The length of the Dniester River is 1352 km; within the territory of Moldova it has a length of 657 km, its catchment representing about 70% of the country's territory. The total length of Prut River is 963 km, on the territory of Moldova it is 695 km (its catchment representing about 24 % of the country's territory). The length of the Danube River on the South part of Moldova is 0,480 km.

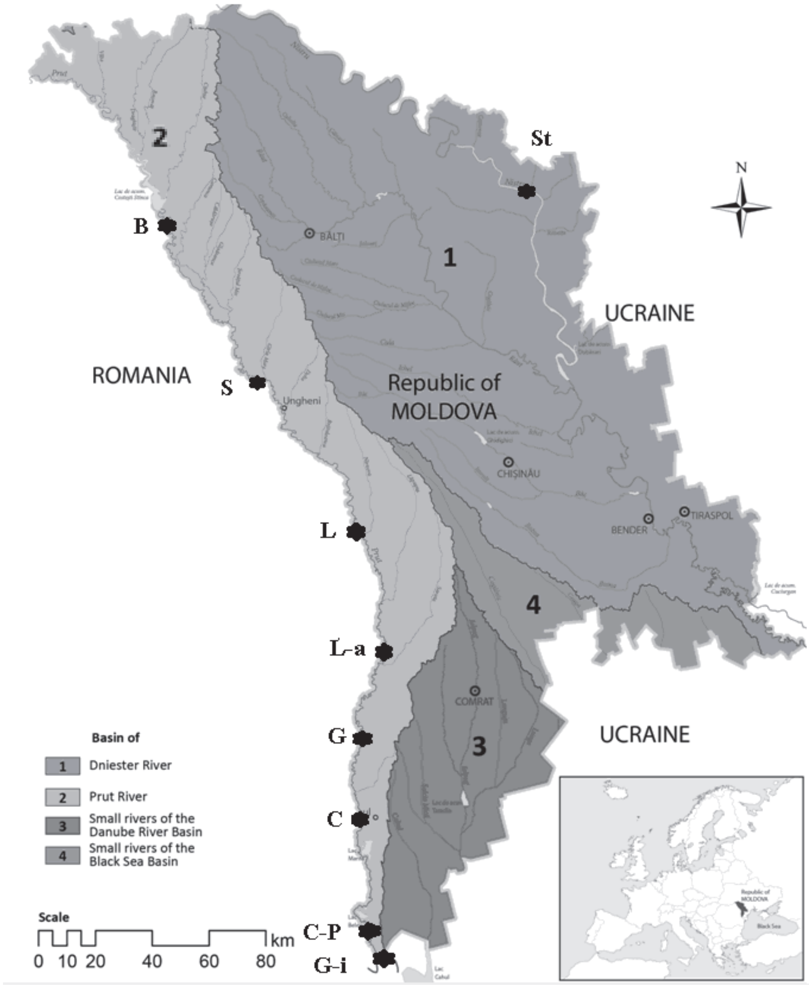

Fig. 1: Map of water basins from Moldova. ✱ = sampling stations where *Palingenia longicauda* has been registered during 2010-2016. Prut River sampling stations from North to South: B: Braniște, S: Sculeni, L: Leușeni, L-a: Leova, G: Gotești, C: Cahul, C-P: Cîșlița-Prut and G-i: Giurgiulești. Dniester River sampling station: St: Stroenți

During 2010-2016 a routine sampling was performed on the main rivers of Moldova: the Dniester River and the Prut River. Supplementary materials from different sampling stations on the Danube River were also collected during 2011-2015. Due to the fact that the

Republic of Moldova borders on Romania to the west and on Ukraine to the north, east and south, all above mentioned rivers are transboundary for Moldova.

The common sampling stations (**CSS**) on the Dniester River: Naslavcea, Volcineţ, Soroca, Camenca, Hîrjău (Erjovo), Goian, Cocieri, Vadul-lui-Vodă, Varniţa, Sucleia, Palanca (Tab. 1.).

The common sampling stations on the Prut River: Costeşti-Stânca reservoir, Branişte, Sculeni, Leuşeni, Leova, Cahul, Cîşliţa-Prut and Giurgiuleşti.

Supplementary sampling stations (**SSP**) on the Dniester River: Stroenţi, Ribnita, Olanesti.

Supplementary sampling stations on the Prut River: Criva, Teţcani, Bădragii Noi, Duruitoarea Nouă, Ungheni, Goteşti, Slobozia Mare.

Supplementary sampling stations (**SSP**) on the Low Danube River: Galati (RO), Giurgiuleşti (MD), Reni (UA), Isaccea (RO), Ismail (UA), Kilia (UA), Vilkovo (UA), Bystroe (UA), Tulcea (RO), Mila 23 (RO), Sulina (RO), Uzlina (RO). RO – Romania, MD – Moldova, UA – Ukraine.

A total number of 14 sampling stations from the Dniester River, 15 from Prut River and one sampling station from Danube River within the present state borders of Moldova were investigated. The total number of supplementary sampling stations situated in Romania and Ukraine was 11.

Sampling was performed with different frequencies: annual, seasonal and monthly. Collecting took place near the river bank at a depth of 0.6-1.5 m and from boats at a depth of 0.5 to 3 m. For quantitative sampling, Petersen and Ekman grabs with an area of capture of 0,025 m^2 and a dredge with an area of capture of 8 m^2 were used. Additionally a hand net for different substrates was used for qualitative sampling. Occasionally material was also collected with a small Petersen grab with a capture area of 10x10 cm and a kick-net with a mesh size of 500 x 500 µm. Samples were preserved by adding 4 % formaldehyde or 70 % ethanol. All individuals have been sorted in the laboratory and identified by using identification keys (Tshernova 1949; Kazlauskas 1977; Kluge 1997).

The identification of species has been carried out with a stereomicroscope МБС-9 and upright microscope Jenaval (Zeiss), as well as with SteREO Discovery.V8 (Zeiss) and upright microscope Axio Imager A.2 (Zeiss). The hydrobiont biomass was determined via weighting on an analytical balance ABS 80-4 Kern to 0.0001g, the material having been previously dried on a paper filter till the disappearance of wet spots. The density and biomass of hydrobionts was converted into ind/m2 and g/m2. Statistical analysis was performed using Statistica V.10, Excel 2007 and 2010 (Microsoft) software.

3 Results

As reflected in table 1, the total number of individuals of *P. longicauda* collected amounted to 578 larvae and 2 adult specimens. The species was found in 9 out of 30 sampling stations. These results could be compared with those described by Russev (1987, table 1), who recorded 1033 larvae and nymphs collected from 22 localities during the period of 1952-1959. During 1956-1973 he found *Palingenia* at 33 localities from a total of 1036 sampling sites along the Bulgarian stretch of the River Danube (Russev 1987, table 2).

The samples of benthic invertebrates from supplementary sampling stations have been analyzed also. However, only isolated mandibles of *P. longicauda* were detected from the Low Danube sampling station Reni (UA). *P. longicauda* larvae and imagines were registered from 8 sampling stations of Prut River and from 1 of Dniester River (Fig. 1)

Occurrence of *P. longicauda* varied from location to location (Tab. 2). The differences may depend on the various ecological conditions: hydromorphological, hydrochemical, type of substrate and level of anthropogenic load.

Tab. 1: Distribution of *P. longicauda* during the years 2010-2016 on the territory of the Republic of Moldova. CSS = common sampling station, SPP = supplementary sampling station

Sampling basin	Locations where P. longicauda was found	Stages	Number of collected individuals
Dniester River	SSP Stroenţi (Stroieşti)	imagines, exuviae	2 adults and 8 exuviae were collected and about 30 flying adults were observed
	CSS Hîrjău (Erjovo), Palanca	isolated mandibles	
Prut River	CSS Braniște, Sculeni, Leușeni, Leova, Cahul, Gotești, Cîșlița-Prut, Giurgiulești	Larvae	578 larvae
	SSP Bădragii Noi	isolated mandibles	

In the sampling stations where *P. longicauda* larvae were recorded, a total number of 462 (quantitative and qualitative) samples of benthic macroinvertebrates were also collected. The frequency of occurrence of *P. longicauda* varied according to the used methods and substrates, from where the samples were collected (Tab. 2).

Tab. 2: Location and occurrence of *Palingenia longicauda* in the Prut River during 2012-2016 on territory of River Moldova. Substrates are arranged in decreasing order of area. N = total number of samples per sampling station, n = number of positive samples including *Palingenia*

Location		Total sampling					Substrates
Sampling stations	Latitude/ Longitude	N	n	%	Dredge %	Grabs %	
Braniste	47˚78'49"/ 27˚25'41"	64	1	2	0	100	gravel, sandy, macrophytes, living parts of terrestrial plants, mud, dead wood
Sculeni	47˚31'80"/ 27˚60'98"	64	8	13	13	87	sandy, mud, gravel, clay, living parts of terrestrial plants, macrophytes
Leuseni	46˚47'32"/ 28˚9'17"	52	17	33	46	54	clay, mud, *Corophium* mud,
Leova	46˚28'13"/ 28˚14'44"	60	18	30	17	83	sandy, mud, clay, gravel,
Cahul	45˚55'16"/ 28˚7'33"	66	5	8	40	60	mud, clay, dead wood
Gotesti	46˚10'37"/ 28˚8'39"	32	6	19	33	67	clay, mud, *Corophium* mud, living parts of terrestrial plants, gravel
Cîșlița-Prut	45˚32'57"/ 28˚9'60"	62	1	2	100	0	clay, mud, sandy
Giurgiulesti	45˚28'20"/ 28˚11'46"	62	1	2	100	0	clay, mud

From table 2 it can be observed that larvae were more often registered at Leuşeni and Leova sampling stations, accounting for 33 and 30 % from total samples, followed by Goteşti sampling station with 19 % frequency of occurrence. Taking into consideration that Goteşti was an additional station and *P. longicauda* was collected both from grab samples as well as dredge samples we consider that such frequency of occurrence is worth of further studies. High density here might be explained by the fact that in these sampling stations there is a large amount of mud, clay and *Corophium* mud (Fig. 2) that are preferred substrates for *P. longicauda* larvae (Landolt et al. 1995). In Leuseni sampling station the density of Corophiidae (Amphipoda) reached 19840 ind/m². Larvae of *Palingenia* were registered only rarely at Braniște, Cîșlița-Prut and Giurgiulești sampling stations, where *P. longicauda* occurred in 2 % of the total number of samples.

This may be caused by a variety of reasons; one of the most important ones are hydromorphological and hydrochemical conditions. The most environmental heterogeneity has been noted at Braniște sampling station. There are many different substrates at this station and only a small area seems suitable for the normal life cycle of *P. longicauda*, but at the same time the highest overall biodiversity of macroinvertebrate species was registered at this station (Munjiu et al. 2014a, Munjiu et al. 2014b). According to results of investigations which have been carried out during May of 2012 - August of 2013, 159 taxa of the benthic macroinvertebrates have been identified, including 20 taxa of Annelida, 22 of Chironomidae, 17 of Crustacea, 12 of Ephemeroptera, 11 of Trichoptera, 21 of Gastropoda, 17 of Bivalvia and 39 taxa of other groups. The species diversity at Branişte station was distinguished by the highest values, 85 species were registered there (Munjiu et al. 2014a).

As regarding the Cîșlița-Prut sampling station, it must be reported that the area near this station is often used by local residents as a pasture. The negative influence of grazing animals is that substances from urine and feces of animals can affect the water quality (Hubbard et al. 2004), and at the same time, grazing can lead to river bank degradation.

The most important factor influencing Giurgiuleşti sampling station is Giurgiuleşti International port, especially the dredging works in the riverbed in this area, that destroy the natural riverbanks.

P. longicauda was found only at sampling stations with steep banks in connection with a small area of mud or clay. These are obviously suitable biotopes for *P. longicauda.* Larvae could only be collected with Petersen and Ekman grab and from dredge samples. Larvae were present in 9 % of quantitative and 4 % of qualitative samples. The combination of grab and dredge sampling was most successful; using Petersen and Ekman grabs with a capture area of 1/40 m^2 it is possible to get the sediments from a bottom depth of up to 10 cm, while the length of U-shaped tubes of larvae reaches 10-25 cm. When a small Petersen grab is used, with a capture area of 10x10 cm^2, it is only possible to reach a depth of up to 5 cm. This depth might not be enough for successfully capturing *P. longicauda* larvae from their tubes. The utilization of a dredge with a capture area of 8 m^2 allows to collect larvae from the bottom surface; this is most effective when the water level is sinking and thus the larvae leave their shelters in the riverbank (as already observed by Csoknya & Ferencz 1972).

The highest abundance of larvae collected by grabs was registered at sampling stations Leuseni: 2520 ind/m^2 (24.07.2013) and Gotesti: 2400 ind/m^2 (20.08.2015), by dredge: 38 in Leuseni (20.03.2013) and 58 specimens in Gotesti (20.08.2015). Imagines could only once be sampled by hand and just about 30 specimens were observed in Stroenti (28.06.2013). The highest mean values of density during 2012-2016, were registered at the following sampling stations. Leuseni: 412 ind/m^2 and Gotesti: 741 ind/m^2. As mentioned above, the substrates preferred by larvae of *P. longicauda* predominate in these sampling stations; that is why the highest values of density were registered there. In the other sampling points the mean density was in Sculeni: 120 ind/m^2, in Leova: 211 ind/m^2 and Cahul: 17 ind/m^2.

It should be noted that there was a high density of *P. longicauda* in the samples collected by grab: 2400 ind/m^2. In the samples collected by dredge on 20.08.2015 in Gotesti there were only 58 larvae. A decrease in the water level here has led to the denudation of part of the riverbank including the larval burrows. All larvae, collected by dredge, were released again into the river after counting.

Fig. 2. Holes of larval burrows of *P. longicauda* on the Prut River (Munjiu O.)

Regarding seasonal dynamics, the period when larvae were most abundant was registered during July-August 2013 and August 2015. During monthly sampling, larvae were registered every month including the winter period of December and February (Fig. 3).

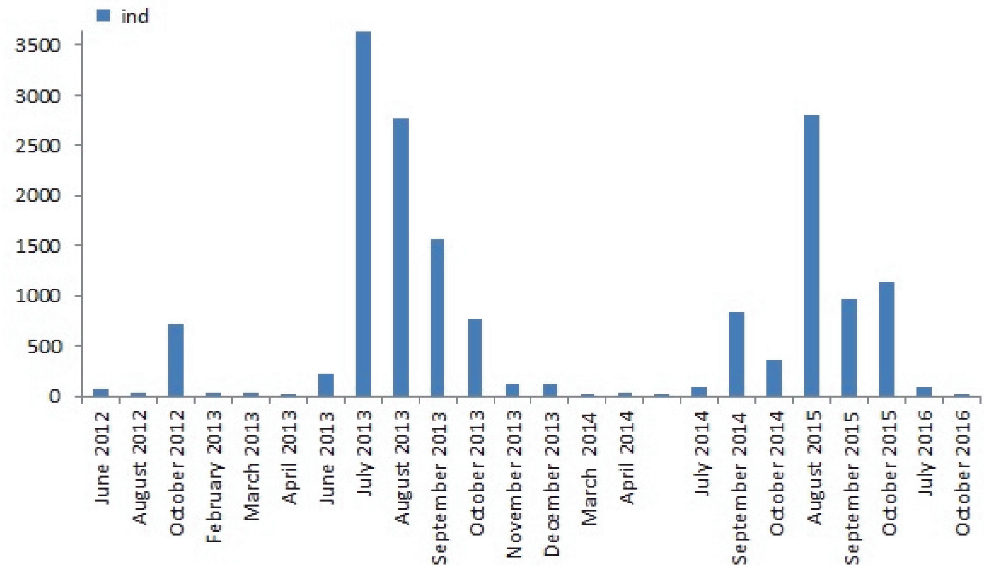

Fig. 3: Seasonal dynamics of *P. longicauda* collected in the Prut River during 2012-2016

The highest biomass of larvae was registered at the end of summer and in early autumn. The maximum value of biomass in quantitative samples was registered at Gotesti, with an amount of 109.36 g/m^2 (20.08.2015) and 106.72 g/m^2 (21.10.2015) correspondingly. Biomass of individuals of *P. longicauda* varied from 0, 0001 g (24.07.2013) up to 0,358 g (16.10.2014).

In comparison to data obtained from neighboring Ukrainian territory in 1970 the abundance of *P. longicauda* larvae, near Vilkovo (Danube River) reached 500-700 ind/m^2, while biomass amounted to 5 g/m^2, which is considerably less than in the Prut River during 2010-2016. Abundance and biomass on the Bulgarian section of the Danube River, during 1956-1973 accounted for an average of 949 ind/m^2 and 269 g/m^2 while biomass was 2.5 times higher than in our investigation.

During the period of this study, larvae of different age classes with different body length were collected (Fig. 4). The length of larvae varied from 5 mm (Leuseni 24.07.2013) to 50 mm (Cahul 05.14). In most cases the big larvae were sampled by dredge on substrate with corophium clay.

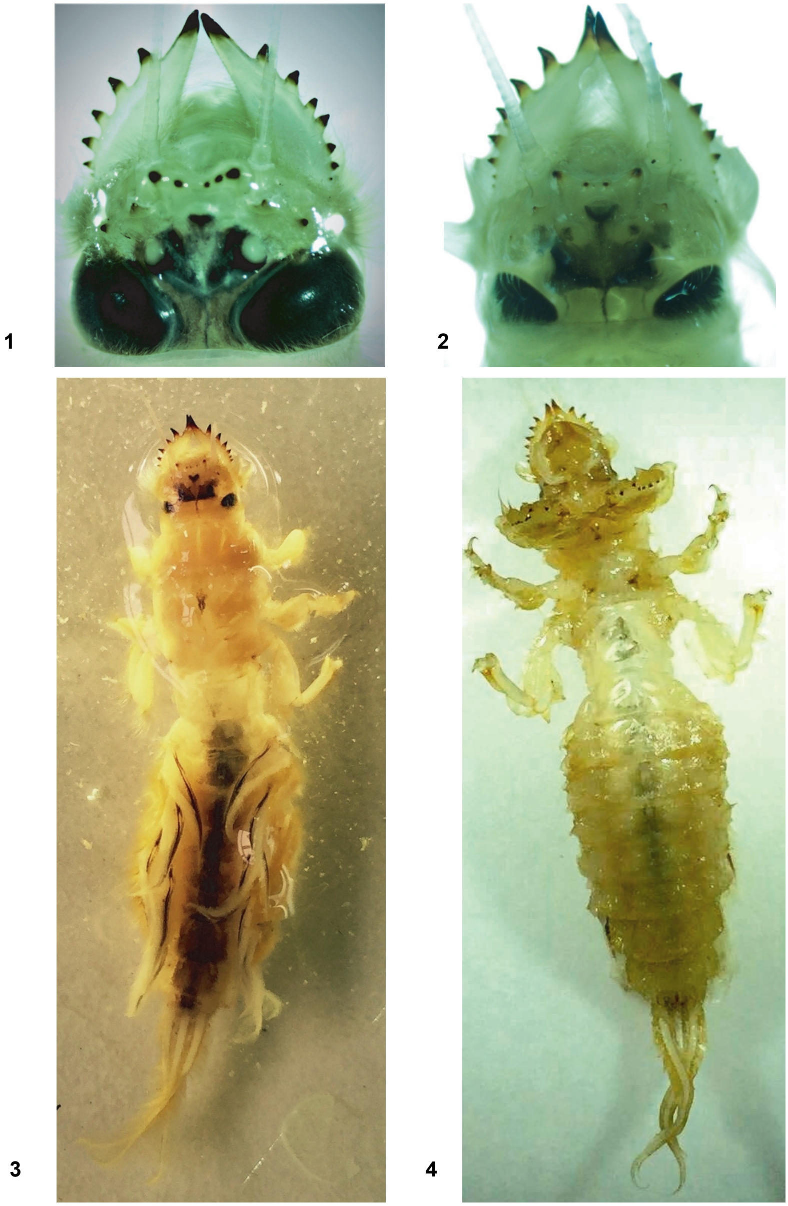

Fig. 4: Larvae of *Palingenia longicauda*, 1) Head of mature larva with compound eyes developed, 2) Head of 2-year larvae, 3) Dorsal view, 4) Ventral view. Photo: Munjiu O.

At Leuseni (20.03.2013) a total of 38 larvae with a length of about 30 mm were registered. Most probably caused by a local short-term discharge of pollutants they were alive but inactive and did not move. In most cases, however, chemical parameters of the Prut River were sufficient for the development of hydrobionts (Zubcov et al. 2014).
According to the investigations carried out during 2013-2014, Prut River waters met the requirements for multifunctional aquatic ecosystems (which may serve as source for drinking water, as well as for irrigation and aquaculture). Dissolved oxygen varied between 59.6-94.6% saturation and water temperature varied from 0.8 to 27.2 ^{0}C. Mineralization values were within the limits of 359-702 mg/l, mineral nitrogen fluctuated between 0.206-2.365 mgN/l (Zubcov et al. 2014).

We should remark that about 30 adult specimens of *P. longicauda* were observed on the water surface at the sampling station Stroentsy (Stroieşti) (Fig. 1) from the Dniester River during the traditional expedition on kayaks performed by Eco-Tiras in June 2013 (Munjiu & Shuberneckij 2013). Such a case was observed only once during 1973-2016, when 11 standard sampling stations on Dniester River were regularly sampled. Only isolated mandibles of this species were sometimes observed in grab samples at Erjova sampling station situated on the upper part of Dubossari reservoir. Two adult specimens and exuviae have been collected from the water surface (Fig. 5).

Fig. 5: Exuviae (shed nymphal skins) and male imagines of *P. longicauda* collected at station Stroenţi (Stroieşti) 06.2013

4 Discussion

During this investigation 578 larvae and 2 adult specimens of *P. longicauda* have been collected. Our results are roughly comparable with data published by Russev (1987), who recorded 1033 larvae and nymphs which had been collected at 22 sampling stations from the Bulgarian River Danube during 1952-1959. Larvae prefer unpolluted large lowland rivers with moderate to fast current velocity and steep clay banks. The same conditions exist on the Middle and Upper sections of the two main rivers of the Republic of Moldova, Dniester and Prut. Due to this fact and also because of the presence of convenient substrate in these rivers, *Palingenia* sp. has been registered as a common species before 1973. Disappearance of this mayfly on the territory of Republic of Moldova, most probably was caused by anthropogenic activity such as hydrotechnical constructions and water pollution as in many European countries (Soldán et al. 2009; Málnás et al. 2011). For example, on the territory of Moldova the Dubossari reservoir was built on the Dniester River, at a distance of 678 km from its mouth in 1954. The Costeşti-Stânca reservoir on the Prut River - at 560 km from the mouth has been built in 1978. These constructions and many other small dams as well led to morphological changes and degradation of riverbeds and negatively influenced the environmental conditions for this sensitive species.

Water pollution as a result of industrialization and widespread use of pesticides (e.g. 22 kg/ha; Bevza et al. 1969) could have lead to the disappearance of *P. longicauda* on the territory of Moldova. On the section of the Prut River situated between Ungheni and Cahul a high level of organic pollution was noted (Bevza et al. 1969), indicated by BOD_{20} (biochemical oxygen demand) 3 times higher than MAC (maximum admissible concentration) and N (NH_4^+) up to 2 mg/l. A similar influence on the water quality was registered from the discharge of pollutants from Bahlui and Jijia, tributaries of the Prut River from Romania, during the past years (Teodosiu et al. 2009). According to the investigations of the Laboratory of Hydrobiology and Ecotoxicology some substances, such as N (NH_4^+) up to 0,49 mg/l (MAC: 0,5N), (NO_2^-) up to 0,034 mg/l (MAC: 0,02) have achieved concentrations of MAC or exceeded this level during 2001-2003 in this section of the Prut River (Munjiu 2006).

The reappearance of *P. longicauda* most probably was connected with the improving water quality from 2000 onwards compared with the previous period. After the adoption of the Water Framework Directive (WFD), Romania performed actions in accordance with the requirements of the Directive, which lead to considerable improvement of the situation on this section of the Prut River in 2006-2007 (Teodosiu 2009).

The rediscovery of *P. longicauda*, since it was considered to have disappeared, took place on 05.11.2010 at Cahul sampling site, in a sample taken by a Petersen grab. This was a larva with a length of about 3 cm and a biomass of 0,1944 g, representing by conform recalculation an abundance of 40 ind/m^2 and biomass of 7.776 g/m^2. Subsequently, data about the reappearance of *P. longicauda* was included in an article about the state of hydrobiocenosis of the Prut River (Zubcov et al. 2014), when at Sculeni, 9.08.2011, *P. longicauda* was registered with a density of 440 ind/m^2 and a biomass of 10,774 g/m^2.

In the Republic of Moldova the restoration of populations obviously was caused by a decrease of industrial activity starting around 1990; in 2013 there was a decrease of about 10 times of wastewater discharge in Prut River compared to 1990 levels (Annuar 2014). Probably some small and inconspicuous populations could survive locally and act hereafter as centers of recolonisation for neighbouring river stretches. Similarly in Hungary *P. longicauda* was rediscovered in the Rába river after 40 years of absence of reports (Kovács & Ambrus 2001). However, a reintroduction project (Tittizer & Andrikovics 2008; Tittizer et al. 2008), on the River Lippe, Germany, failed (Tittizer in Kullmann 2015: 105, footnote 299).

Some peculiarities of *P. longicauda* populations can presumably contribute to their survival as, for example, eggs of a considerable part of females may develop parthenogenetically (Landolt et al. 1997; Andrikovics & Turcsányi 2001), this being relevant for small populations. Also genetic variability in small populations can be surprisingly high, which should equally contribute to the survival of *P. longicauda* (Bálint et al., 2012).

The results of this investigation clearly indicate the existence of at least some vital populations of the endangered mayfly *P. longicauda* on the territory of the Republic of Moldova and thereby confirm a new significant area of refuge for *P. longicauda* in Europe.

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